THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 55, No. 5

Printed in U.S.A.

STRATUM GRANULOSUM: DISSECTION FROM CATTLE HOOF EPIDERMIS*

ARTHUR R. UGEL, M.D. AND WILLIAM IDLER, B.S.

ABSTRACT

A hairless area of epidermis is present on the posterior aspect of cattle hooves. When this area is excised the cut surfaces reveal three thick and well-demarcated cellular layers: 1) a glassy-clear stratum corneum; 2) the underlying epidermal cell layers; 3) the dermis. Microscopically, the stratum granulosum of this tissue averages 20 cells in thickness and is free of hair follicles, sebaceous and sweat glands. In view of the thickness of these cellular layers and the sharp demarcation between the stratum corneum and granular cell layers, it is possible by fine dissection to obtain sheets of fresh tissue which contain large amounts of keratohyalin. Such preparations are contaminated with variable amounts of cells from the stratum malpighii and dermal ridges, and small amounts of cornified and basal cells.

Morphological changes occurring in the epidermis define the basal, spinous, granular and cornified cell layers. If cells composing a particular cell layer could be isolated, then additional experimental approaches designed to determine the biochemical events related to these morphological events might be feasible. This communication describes a tissue, cattle hoof epidermis, which is unique in that the granular cell layer is sufficiently thick to permit its partial separation by fine dissection.

MATERIALS AND METHODS

Adult cream-colored hooves were obtained from a local abattoir at the time of slaughter and maintained at 4° C (Fig. 1).

Hooves were scrubbed clean with a brush using cold tap water. Segments of tissue 2 cm × 4 cm were excised from just beneath the posterior hairline (Fig. 2A). Segments were then sliced into blocks 1.0 cm thick, trimmed of excess stratum corneum and dermis, and then cut into approximately 1.0 mm thick slices (Fig. 2B). Slices were laid flat under a dissecting microscope and all tissue lying beneath the stratum corneum except for a very fine margin (approximately 0.5 mm thick) of opalescent tissue was excised and discarded (Fig. 2B, 2C). Finally, with the aid of a dissecting microscope the opalescent margin was excised, care being taken not to include stratum corneum (Fig. 2C, 2D).

For light microscopy, specimens were fixed in 80 percent methanol and stained with Congo red, Harris' hematoxylin or Pauly's reagent as previously described (1).

Received April 24, 1970; accepted for publication June 20, 1970.

* From the Dermatology Branch, National Cancer Institute, Bethesda, Maryland 20014.

RESULTS

Gross anatomy. Excised segments reveal three thick and well demarcated cellular layers (Fig. 2, 3, 4): 1) a glassy-clear stratum corneum; 2) the underlying epidermal cell layers; 3) the dermis. The stratum corneum gradually increases in thickness toward the plantar surface of the hoof; at the superior margin of the excised segments the stratum corneum averages 1.0 mm in thickness (Fig. 2, 3), and increases to 6.0 mm in thickness at the inferior margin (Fig. 2, 4). The underlying epidermal cell layers remain fairly constant in thickness (1.2 mm to 1.6 mm) at both superior and inferior margins of the excised segments (Fig. 3, 4).

Certain hooves demonstrate melanotic streaks extending from the posterior hairline to the base of the hoof. When excised, the origin of these streaks can be traced to the basal cell area at the superior margin of the excised segments, and inferior to this, pigment is found only in the stratum corneum (Fig. 5).

Histology. Sections of non-dissected tissue reveal a thick stratum corneum. The stratum granulosum is greatly hypertrophied, averaging 20 cells in thickness, and ranging from 10 cells to 60 cells (Fig. 6). Keratohyalin granules of hoof epidermis are hematoxylin, Pauly, and Congo red positive. The stratum malpighii and stratum basale are unremarkable, except for the prominent dermal ridges in these layers. No hair follicles, sebaceous or sweat glands are present.

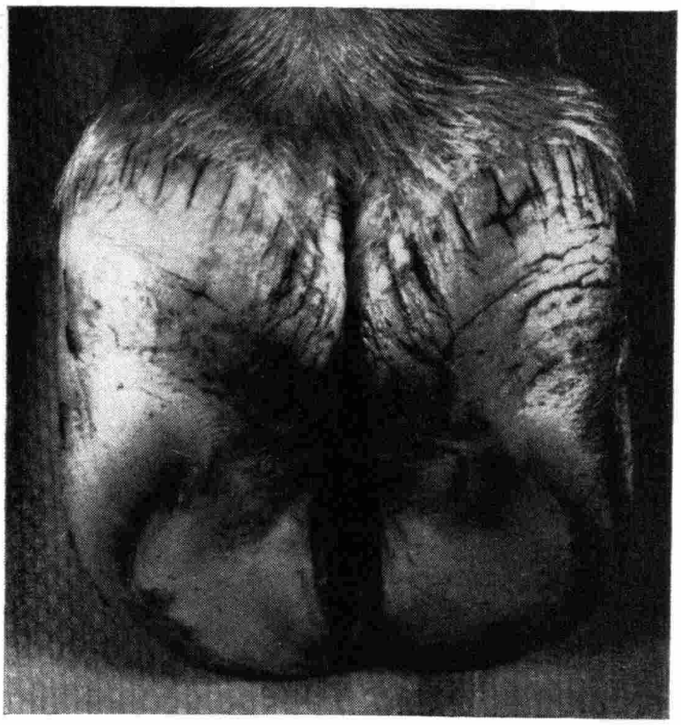

FIG. 1. Posterior view of adult cream-colored cattle hoof.

Dissected tissue. Finely dissected specimens obtained as described in Methods are composed of sheets of stratum granulosum, stratum malpighii, stratum corneum and stratum basale. Distribution of cells for 12 different preparations was estimated by counting 10 random fields for each preparation in the light microscope at a magnification of 100. Cells con-

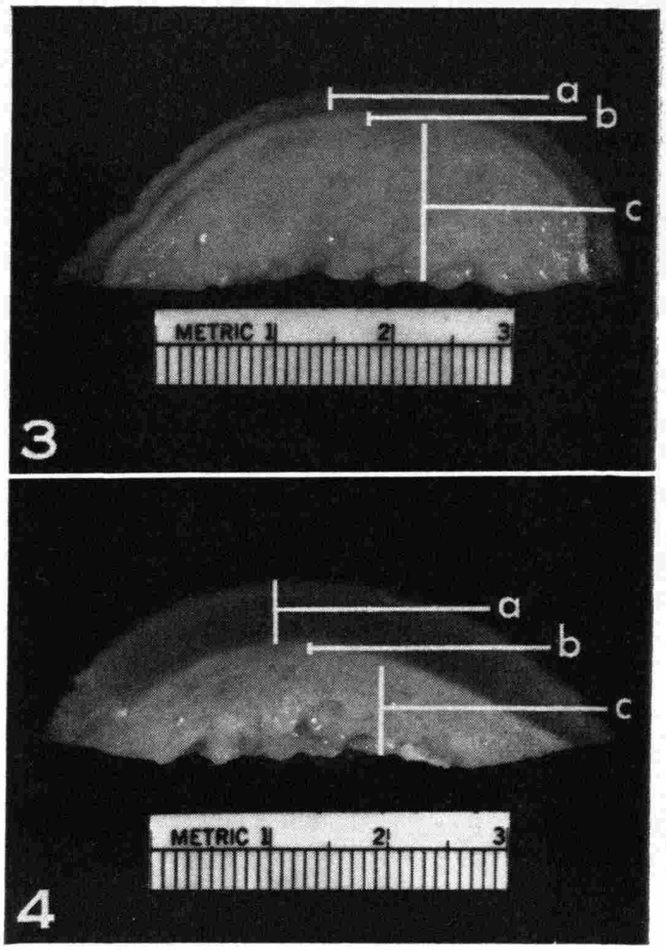

FIG. 3, 4. Superior margin (Fig. 3) and inferior margin (Fig. 4) of excised segment revealing three distinct cellular layers: a) stratum corneum; b) underlying epidermal cell layers; c) dermis. The stratum corneum is approximately 2.0 mm thick at the superior margin and 6.0 mm thick at the inferior margin.

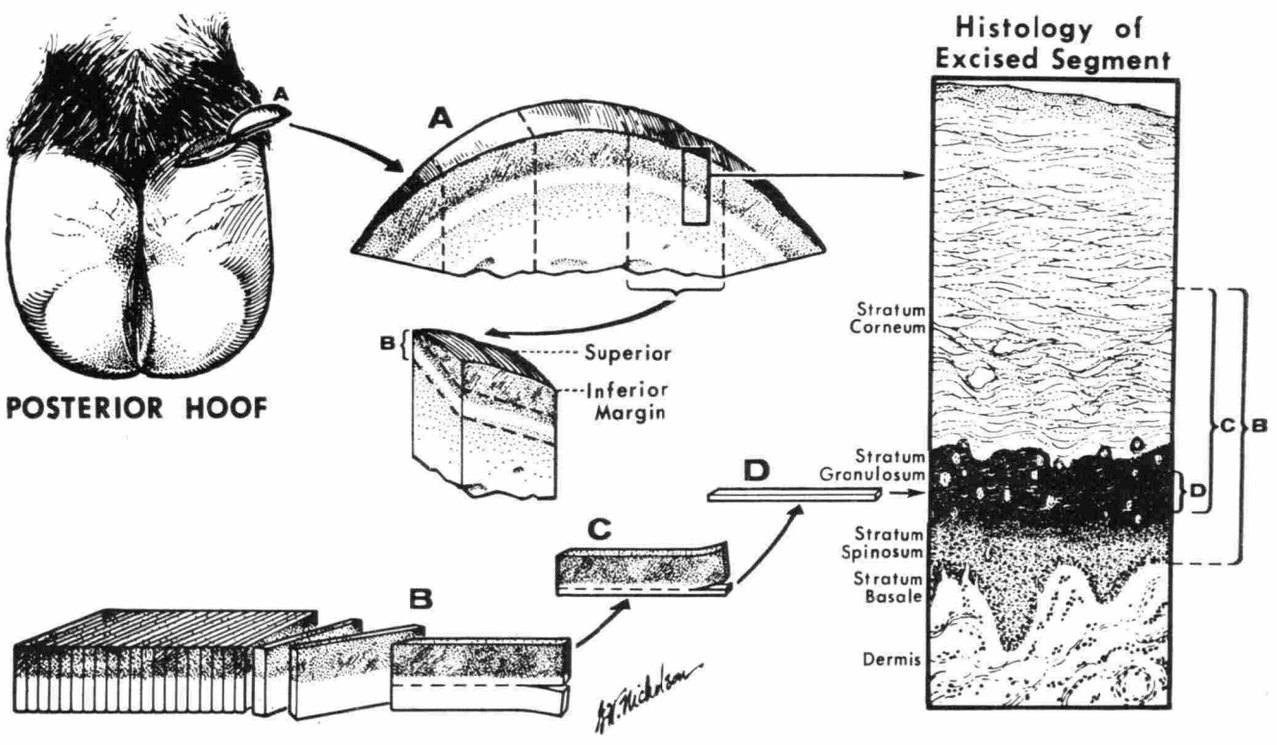

FIG. 2. See Methods

taining keratohyalin granules ranged from 29 percent to 86 percent per preparation, and averaged 42 percent for all 12 preparations. Major contaminants are due to cells of the stratum malpighii and dermal ridges, and small amounts of cornified cells and basal cells (Figs. 7–9).

DISCUSSION

Cattle hoof epidermis has certain advantages for obtaining epidermis for study. The tissue is extremely thick and fresh specimens reveal three well demarcated layers. Because of the thickness and demarcation, separation of hoof epidermis into stratum corneum, underlying viable epidermis and dermis is easily performed, and permits the use of fresh untreated specimens. Further, the absence of hair follicles, sweat and sebaceous glands eliminates these organs as contaminants. Cattle snout is also a thick epidermal tissue which can be dissected into layers, but the stratum granulosum of snout is relatively thin (6). Other tissue sources such as rat, guinea pig, or human epidermis are too thin to permit dissection, and require the use of physical manipulations involving stretching (8), scraping (5), heating (1), the use of trypsin (3, 4) or the use of salt solutions (1–3) in order to separate the epidermis from the dermis. Further purification of the stratum granulosum in these thin tissues has required the use of ethylenediaminetetraacetic acid or tetraphenylboron (3).

The stratum granulosum of hoof epidermis is unusually thick (20 cells) when compared to other tissues such a man, rat, or guinea pig which have a granular layer that is only three to five cells thick. Attempts to further

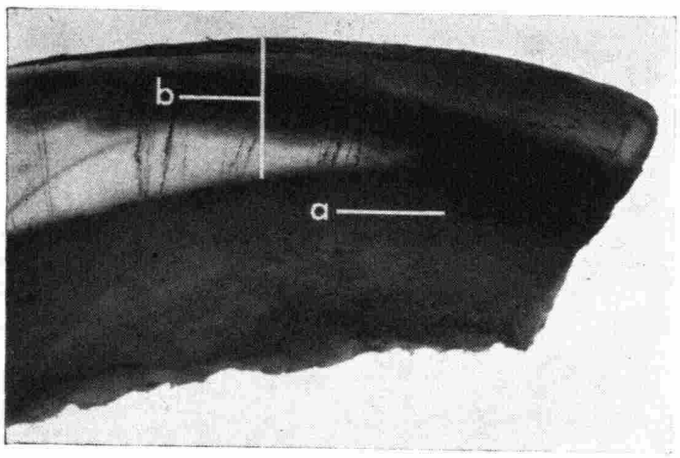

FIG. 5. Vertical segment of posterior hoof revealing melanotic streak with origin in basal cell layer of superior margin (a) and extending downward in stratum corneum (b).

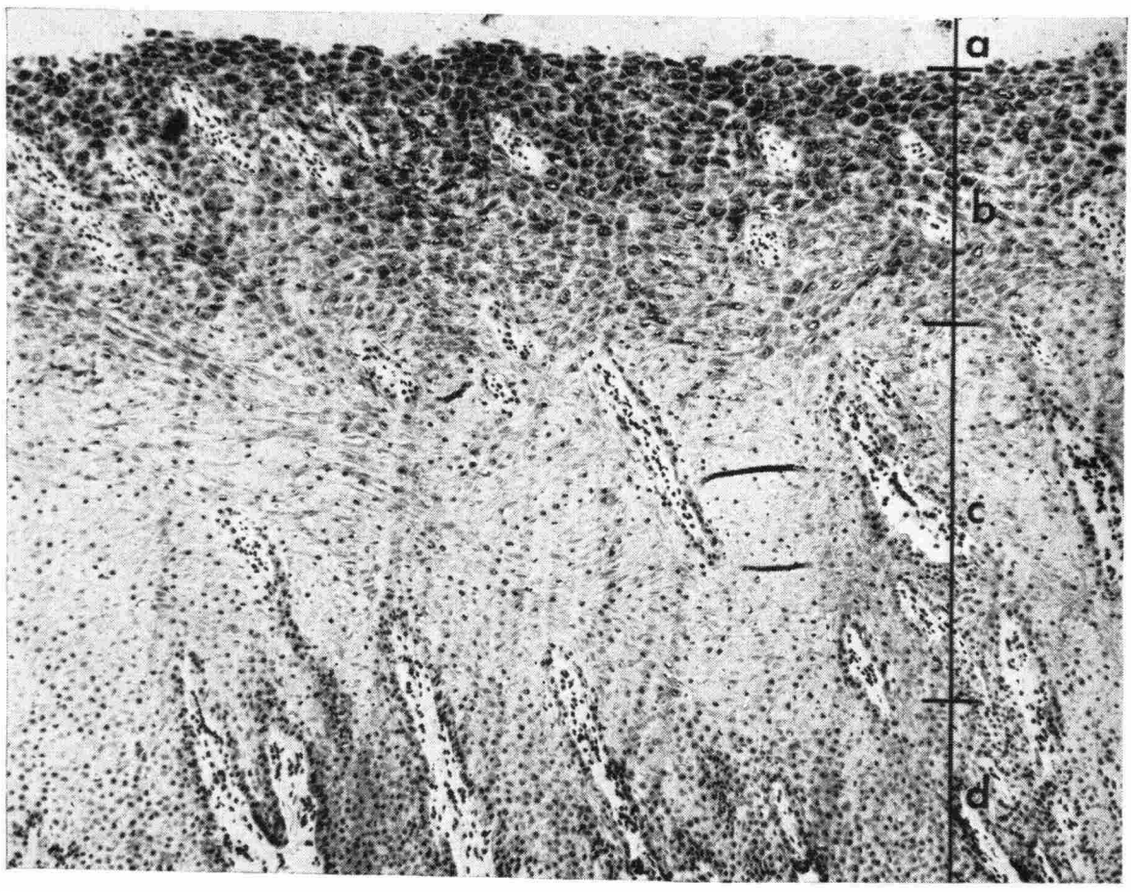

FIG. 6. Section of hoof epidermis revealing a) lowermost stratum corneum, b) thick stratum granulosum, c) stratum spinosum, d) basal cell area. Hematoxylin × 40.

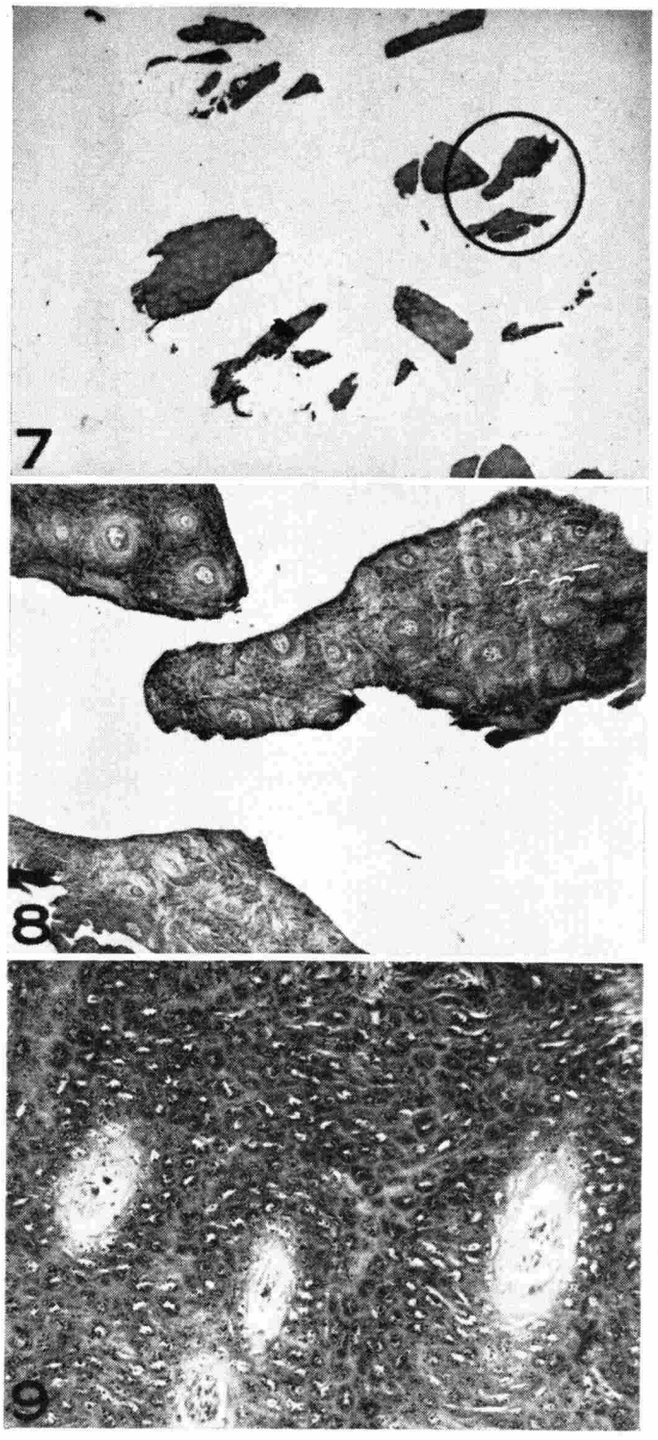

FIG. 7. Dissected tissue slices. H & E × 5.
FIG. 8. Higher magnification of Fig. 7. Tissue contains large amounts of keratohyalin. H & E × 40.
FIG. 9. Higher magnification of Fig. 7 which better demonstrates the numerous keratohyalin granules. H & E × 160.

separate the stratum granulosum of hoof epidermis by fine dissection have resulted in large fluctuations in the percentage of cells containing keratohyalin granules (29 to 86%). However, the classification of separated layers on a purely morphological basis is arbitrary and probably very imprecise with respect to biochemical events which no doubt antecede morphological markers. The method of preparing fresh tissue has proven useful for studies on keratohyalin granules (7).

Gross observations upon the area of hoof described in this communication indicate that this site may be part of the generative matrix for portions of the hoof. These observations are: 1) the gradual increase in the thickness of the stratum corneum from superior to inferior margins of the excised segments; 2) the occasional presence of melanotic streaks having their origin in the basal cell layer at the superior margin of the excised segments and extending to the base of the hoof in the stratum corneum. Such observations indicate that cells originating at the excised site travel outward as well as downward. As such, this matrix would generate and maintain a large epidermal structure, and would explain the very thick epidermis found at the excised area. In this connection, the tissue described in this communication may be related to hard keratin (hair, horn, nails) rather than to soft keratin (epidermis, palate) (4, 6). However, the tissue is a stratifying epidermis, having the classical layers and the cellular components (keratohyalin granules, tonofilaments) appear typical at the ultrastructural level (7).

I thank B. Sanders and B. Coolidge for technical assistance.

REFERENCES

1. Baumberger, J. P. and Suntzeff, V.: Methods for the separation of epidermis from dermis and some physiologic and chemical properties of isolated epidermis. J. Nat. Cancer Inst., *2:* 413, 1941.
2. Felsher, Z.: Studies on the adherence of epidermis to the corium. J. Invest. Derm., *8:* 35, 1947.
3. Gumucia, J., Feldkamp, C. and Bernstein, I. A.: Studies on localization of "histidine-rich" peptide material present in epidermis of the newborn rat. J. Invest. Derm., *49:* 545, 1967.
4. Matoltsy, A. G.: Mechanism of keratinization, pp. 1–25, *Fundamentals of Keratinization.* Eds., Butcher E. O. and Sognnaes. R. F. Amer. Assoc. Adv. Sci. Publ. No. 70, 1962.
5. Rosett, T., Burk, P., Ohkido, M., Smith, J. G. and Yardley, H. J.: Stretch separation of epidermis. J. Invest. Derm., *49:* 15, 1967.
6. Rudall, K. M.: The proteins of the mammalian epidermis. Advances Prot. Chem., *7:* 253, 1952.
7. Ugel, A. R.: Keratohyalin: extraction and *in vitro* aggregation. Science, *166:* 250, 1969.
8. Van Scott, E. J.: Mechanical separation of the epidermis from the corium. J. Invest. Derm., *18:* 377, 1952.